to the native membrane environment. To improve the solubilization of membrane proteins (MPs) and allow their study in bicellar systems, D6PC was replaced by detergents from the monoalkylphosphocholine (MAPCHO) family of which dodecylphosphocholine (DPC) is known for its ability to solubilize MPs. More specifically DPC, tetradecyl- (TPC) and hexadecylPC (HPC) have been employed. To verify the possibility of making bicelles with different hydrophobic thickness to better accommodate MPs, D14PC was also replaced by phospholipids with different acyl chain lengths: dilauroylPC (D12PC), dipalmitoylPC (D16PC) and distearoylPC (D18PC). Preliminary results obtained by 31P solid-state NMR at several lipid-to-detergent molar ratios (q) and temperatures indicate that magnetically-oriented bicelles can be formed with D12PC/DPC (q=2, 17-47°C), D14PC/DPC (q=2-3, 32-52°C), D16PC/DPC (q=1.6-2.4, 42-47°C), D14PC/TPC (q=2, 32-57°C) and D16PC/TPC (q=1.6-2.4, 42-52°C). The temperature range at which these bicelles orient is, thus, dictated by the gel-to-fluid phase transition temperature of the phospholipids. Moreover, the longer the phospholipid chain length, the smaller the q ratio range at which bicelles orient. These results will be discussed in terms of PCs and MAPCHOs solubility. These are promising model membranes that could be amenable to both solution- and solid-state NMR, thus enabling structure determination with different bilayer thickness as well as the study of lipid interactions with a single membrane mimicking system.

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Fluorescence Measurements of Aromatic Amino Acids in the Presence of Lipid Membranes

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Amphiphilic peptides are capable of finding their way to, and occasionally through, cellular membranes using a mechanism that includes specific amino acid sequences. Physical measurements of amino acid-lipid interactions are of interest for a quantitative description of peptide affinities to biological membranes. In this study, we investigate small peptide-lipid interactions using the fluorescence of the aromatic amino acids tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe). Reference spectra in isopropanol-water, ethylene-glycol-water, DMSO-water, and polyethyleneglycol-water solutions are obtain to mimic hydrophobic environments and are used to quantify the interaction of Lys-Tyr-Lys, Trp-Gly, and Gly-Phe with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS) lipid membranes. These fluorescence data complement previously reported UV absorption data and have the advantage of eliminating background and scatter from solution. Together with NMR data, these results can be used to more fully characterize lipid-aromatic amino residue interactions.

2598-Pos Board B290

Single Molecule Studies of PKCα Activation Mechanism on Membrane Surfaces

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The master kinase PKCα is a central player in the normal function of many signaling pathways, and also plays a role in multiple pathologies. The conventional model for activation of classical PKCs, including PKCα, proposes two major steps during conversion of inactive, cytoplasmic enzyme to active, membrane-bound enzyme. First, a cytoplasmic Ca^{2+} signal activates C2 domain that serves to recruit the inactive enzyme from cytoplasm to the PS-rich inner leaflet of plasma membrane. Second, inactive enzyme, bound to plasma membrane via its C2 domain, is activated by appearance of the second messenger lipid diacylglycerol (DAG) that recruits the inhibitory C1A and C1B domains from the kinase domain to the membrane, thus activating the kinase. To test the predictions of this model, we have employed single molecule methods to analyze membrane binding and surface diffusion of full length PKCα, and also simpler constructs containing a subset of its domains. TIRF microscopy was used to visualize these proteins on supported lipid bilayers containing different combinations of target lipids in order to investigate the contributions of individual domains to membrane binding and lipid-induced kinase activation. Previous studies have shown that single molecule TIRF analysis of peripheral proteins on supported lipid bilayers provides extensive information on protein-lipid interactions difficult to obtain by other methods (Ziemba, Knight & Falke (2012) Biochemistry 51(8):1638-1647. Knight, Lerner, Marcano-Velazquez, Pastor & Falke (2010) Biophys J 99:2879-87). The present single molecule analysis reveals a previously unknown intermediate in the PKCα activation reaction, and indicates this is the major intermediate while the enzyme awaits the appearance of activating diacylglycerol or phorbol ester. The findings yield new insights into the PKCα activation mechanism, and show that the single molecule approach provides a new window into the activation mechanism of membrane-bound signaling enzymes.

2599-Pos Board B291

The Ebola Virus Matrix Protein Bends Biological Membranes

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Ebola is one of the most virulent pathogen that causes severe hemorrhagic fever with fatality rate as high as 90%. There is an urgency in the development of treatment as there are no known drugs or vaccines approved by the FDA and the virus poses a serious health and potential biological threat. The Ebola viral matrix protein 40 (VP40), is the most abundantly express protein of the viurs and alone harbors the ability to form virus like particles (VLPs) that are indistinguishable from the authentic virus. The mechanism of VP40 assembly at the plasma membrane before the release of the virions remains poorly understood. In order to better understand the process of viral egress, it is crucial to understand how VP40 is able to bend the plasma membrane to regulate formation of VLPs. Here we take a detailed look at how VP40 alone is able to bend giant unilamellar vesicles (GUVs) membranes, a model for VLP egress. Here, we imaged GUV's containing PC:PE with and without anionic lipids enriched in the plasma membrane such as PS and phosphoinositides to determine VP40 budding selectivity. The results demonstrate PS-dependent bending and vesiculation from GUV's membrane. The vesiculation is not enhanced in the presence of cholesterol and completely inhibited in GUVs composed of polyvalent phosphoinositides. In concert with budding from live human cell models, the bending and VLP formation is unique to PS containing membranes. GUVs have previously been utilized to understand membrane scission and to expose the role of viral proteins in membrane bending. Elucidating the effect of VP40 on GUVs composed of lipids that mimic biological membranes will help understand the mechanistic details of an otherwise illusive membrane remodeler.

2600-Pos Board B292

Palmitoylation as a Key Factor to Understand Sp-C-Lipid Interactions in the Lung Surfactant System

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Surfactant protein C (SP-C) has been regarded as the most specific protein linked to lung development. So far, great efforts have been done to understand the structure-function relationships of this lipopeptide, nevertheless its high hydrophobicity and tendency to aggregate forming amyloid-like structures have made its study a challenging task. Previous evidence has pointed out the importance of SP-C palmitoylation in sustaining the proper dynamics of lung surfactant, but the mechanism by which this posttranslational modification stabilizes the interfacial surfactant film under dynamic compression-expansion cycles mimicking the process of breathing, is still unrevealed. In this work we have compared the behavior of a native palmitoylated SP-C with a non-palmitoylated recombinant SP-C (rSP-C) in membrane environments by means of ATR-FTIR spectroscopy. Our results suggest that palmitoylation modulates SP-C-lipid interactions and besides, it may play a dual role in combination with electrostatic interactions with anionic phospholipids (POPG) to maintain a proper SP-C conformation in the lung surfactant context. Functional approaches will provide further insights into SP-C palmitoylation-induced effects, allowing the characterization of SP-C structure-function determinants.

2601-Pos Board B293

Clarifying the Roles of Cardiolipin

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Cardiolipins (CL) are uniquely structured double lipids that are universally found in membranes which couple electron transport and phosphorylation. The proposed roles of CL in these membranes include mainly two aspects: the effects on the structure and dynamics of the membranes' lipid component and the interplay with membrane-associated proteins. In previous work, we have studied both of these aspects by utilizing atomistic molecular dynamics simulations, starting with the general effects of CL on membrane properties and the interplay with ions [1], further moving on to the interactions with the

membrane-embedded respiratory complex cyt bc1 [2]. The latter model system that is of greater interest comprises the entire cyt bc1 dimer of the purple photosynthetic bacterium Rhodobacter capsulatus embedded in a lipid bilayer, whose lipid composition mimics that of the inner mitochondrial membrane. Intriguingly CLs were observed to diffuse spontaneously to the dimer interface and to the immediate vicinity of the catalytic Qi-sites [2]. This observation is in agreement with experimental data, as CLs are indeed located close to the Qi-sites in several X-ray crystal structures of the complex. Importantly, our observations support the proposed role of CL in delivering protons for the non-reduced substrate forms in the active site.
In ongoing work that we discuss here we focus more specifically on the roles of individual components of the proposed proton uptake pathway (CL, water, and individual protein residues) and on the atom-level reaction mechanism in the binding pocket. To this end, further MD simulations, QM calculations, and cite-directed mutagenesis experiments were employed. We also discuss whether there is a plausible pathway for substrate movement between the active sites through the lipid-filled insides of the complex, and the role of oxidative stress in cyt bc1 behavior.
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2602-Pos Board B294
Fluorescence Correlation Spectroscopy Reveals Additional Information on Protein Insertions into Phospholipid Monolayers
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The generation of curvature in phospholipid membranes is a crucial step in vesicle and tube formation. A variety of proteins in eukaryotic cells are able to induce such curved membrane structures and produce coated transport vesicles or tubular carriers. Membrane curvature can be generated in different ways. Sar1, which is a small GTPase of the RAS superfamily and a part of the coat protein complex II (COPII), and the N-BAR dimer of amphiphysin II, which is involved in clathrin-coated vesicle formation, both have amphipathic α-helices, which insert only into the proximal leaflet of a phospholipid membrane. Phospholipid monolayers are therefore convenient model systems for analyzing the protein-lipid interactions.
While a typical Langmuir film balance setup allows to observe the interaction of proteins with phospholipid monolayers (by changing the surface pressure or the surface area),the determination of protein concentrations and diffusion properties is not possible.
To overcome these limitations, we combine a Langmuir film balance with a confocal fluorescence correlation spectroscopy setup for optical detection. This setup, which was described for the first time by Gudmand et.al (Biophys. J., 2009, 96, 4598-4609), allows FCS measurements in a monolayer system.
Here we show some basic interaction studies of Sar1 and N-BAR with phospholipid monolayers at different surface pressures. In all cases, FCS measurements were carried out to obtain additional information on the protein.

2603-Pos Board B295
Membrane Phosphoinositide Turnover by Voltage Sensing Phosphatases
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The PI levels can be controlled by the state of dynamic equilibrium between lipid kinase-induced phosphorylation and lipid phosphatase-induced dephosphorylation. The voltage sensing phosphatases (VSPs) gain enzymatic activity upon membrane depolarization so they can trigger temporary imbalance of PIs by electrophysiological method. Recent studies reported that VSPs have both 3- and 5- phosphatase activities upon membrane depolarization. However, its enzymatic characteristics and mechanism are still unclear. Here we examined the functional role of Dr-VSP and Ci-VSP fused with PTEN (Ci-VSPTEN) in the PI turnover through Fluorescence Resonance Energy Transfer (FRET) using Pleckstrin Homology (PH) domains. VSP-induced turnover of diverse PIs, such as $PI(3,4)P_2$, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ showed different kinetics in degradation and resynthesis. When the Dr-VSP expressing cells were given with a 120 mV/3 s of depolarization, the level of $PI(4,5)P_2$, indicated by PH(PLCδ) FRET fairs, decreased rapidly ($\tau = 1.18 \pm 0.17$ sec), while the levels of $PI(3,4)P_2$ and $PI(3,4,5)P_3$, indicated by PH(Akt), decreased slowly ($\tau = 1.36 \pm 0.07$ sec) and incompletely. The voltage dependent enzymatic activity of phosphatases was also investigated by addressing ramp depolarization which increases from 0 mV to 120 mV for 20 s (holding potential = −80 mV). PH(PLCδ) FRET started to decrease from the voltage of 33.0 ± 6.9 mV, whereas PH(Akt) FRET began to decrease from 71.0 ± 11 mV. In addition, recovery of $PI(4,5)P_2$ and $PI(3,4)P_2$ /$PI(3,4,5)P_3$ showed distinct phenomena in time constants (τ_{rev} = 7.17 ± 1.46 sec and 57.46 ± 4.78 sec, respectively) and in absolute quantity (92.3 ± 8.0 % and 55.3 ± 2.4 %, respectively). Thus, our results suggest that VSP can act as a dual phosphatase and deplete PIs with different time constants. The data also provide the molecular properties of endogenous PI resynthesis in living cells.

2604-Pos Board B296
Toward Understanding the Role of Amot130 Lipid Binding in Cellular Proliferation and Migration
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Background: Amots are adaptor proteins which coordinate signaling that controls cellular differentiation and proliferation. Amot proteins have a novel lipid binding domain, the Amot coiled-coil homology (ACCH) domain, which selectively binds monophosphorylated phosphatidylinositols (PI) and targets transcription factors to the nucleus. Understanding the biophysical mechanisms of lipid binding may provide pathways to modulate protein sorting and downstream signaling events inducing cellular differentiation, cancer cell proliferation, and migration. So far, all work reported on signaling based on Amot expression fails to distinguish between the role of the Amot80 and the 130 family members as they share a common ACCH domain.
Objective: The goal of this project is to specifically associate the Amot130 ACCH lipid binding with function related to ductal hyperplasia and breast cancer phenotypes.
Method: Mutations were carried forward based on lipid sedimentation, FRET, and SAXS assays against the ACCH domain. Site-directed mutagenesis was employed to probe the specific contributions of 7 selected lysines and arginines toward lipid head-group binding in the full length protein. Target proteins will be fluoresced to determine whether they retain their ability of binding to membrane. Cells fractionation will be used to quantify the protein amount that has passed the nuclear membrane.
Amot family members bind core polarity proteins controlling the apical domain organization of epithelial cells; and Yap, a transcriptional co-activator that regulates cell growth. Mutations in Amot affecting lipid binding to the apical membrane lead to disability to control cell growth and differentiation. Consequently, abnormal phenotypic changes regarding cell migration and polarization will be observed when growing cells on matrigel assays. Such mutations can also interfere with Amot binding to Yap, results in unregulated cell growth and proliferation. Yap expression level will be measured using western blots and immunoprecipitation techniques.

2605-Pos Board B297
Characterizing Pulmonary Surfactant Peptide and Lipid Interactions with Various Spectroscopic Techniques
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In vitro studies of pulmonary surfactant peptides in vesicles of mixed lipid composition are examined using nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) and fluorescence spectroscopy. As KL_4, a 21 residue mimetic of surfactant protein B (SP-B), has been used to restore lung compliance and promote gaseous exchange in premature babies with respiratory distress syndrome (RDS); and has served as a model in elucidating the mechanism by which lipids are trafficked to and from the air-fluid interface of alveoli. Here we utilize a pyrene phospholipid analog to investigate the effect of KL_4 on lipid organization and acyl chain dynamics by monitoring changes in excimer-to-monomer (I_e/I_m) ratio. This experiment probes the environment of the hydrophobic core of DPPC/POPG and POPC/POPG liposomes. An average decrease of ~27-40% and ~0-10% in I_e/I_m was observed in the DPPC/POPG and POPC/POPG LUVs, respectively, with increasing peptide concentration (0.5 to 5 mol%). This decrease is directly proportional to a lowered probability of excimer formation, which is highly dependent on proximal interactions of an excited monomer with a pyrene moiety at ground state. The ability of the peptide to modify membrane fluidity properties was studied via anisotropy measurements of a rhodamine-labeled phospholipid. A steady increase in the order was observed in the DPPC/POPG liposomes with relatively constant fluorescence intensity, while collisional quenching was observed in the POPC/POPG liposomes. Further studies were performed on the SP-B N and C-terminal constructs for correlation with NMR and EPR observations, and proposed mechanisms of peptide-mediated lipid trafficking.